Sugars, sugar alcohols, fruit acids, and ascorbic acid in wild Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis*) with special reference to influence of latitude and altitude

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ABSTRACT

Wild berries of *Hippophaë rhamnoides* ssp. *sinensis* were collected from nine natural growth sites in China in three consecutive years in order to get an overall profile of the sugars, sugar alcohols, fruit acids, and ascorbic acid, and especially of the influence of the latitude and altitude of the growth place on these components. The contents of fructose, glucose, and L-quebrachitol in the berry juice varied in the ranges of 0.01–7.17, 0.05–7.85 and 0.21–1.09 g/100 mL, respectively, those of malic, quinic, and ascorbic acids were 1.55–8.84, 0.07–2.94, and 0.25–1.66 g/100 mL, respectively. The berries from Hebei and Inner Mongolia were characterized by high contents of sugars and L-quebrachitol and low contents of malic acid and ascorbic acid. In contrast, the berries from Sichuan and Qinghai contained lower contents of sugars and higher contents of malic acid and ascorbic acid than the berries from other growth areas. The berries from Sichuan differed considerably from others by the remarkably low contents of sugars and the exceptionally high contents of acids. The contents of fructose, glucose, and total sugar decreased as the altitude increased and as the latitude decreased ($p < 0.05$). In contrast, the contents of malic acid and ascorbic acid increased as the altitude increased and as the latitude decreased ($p < 0.05$). The contents of quinic acid and L-quebrachitol correlated strongly and positively with the latitude ($p < 0.01$).

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1. Introduction

Hippophaë rhamnoides ssp. *sinensis* is an important natural resource in China. It is used as a pioneer plant with a significant value for water and soil conservation because of its strong roots with nitrogen fixing ability as well as its resistance to extreme conditions such as drought, cold, and salinity. Sea buckthorn berry has been applied as an ingredient in traditional Chinese medicine since the ancient times. The berry and berry fractions have beneficial effects on the skin (Upadhyay et al., 2009; Yang et al., 1999, 2000), mucosa (Xing et al., 2002), eyes (Larmo et al., 2010), and the cardiovascular system (Basu et al., 2007; Johansson, Korte, Yang, Stanley, & Kallio, 2000; Larmo, Alin, Salminen, Kallio, & Tahvonen, 2008). Antioxidative activities (Geetha, Ram, Singh, Ilavazhagan, & Sawhney, 2002; Shukla et al., 2006) and positive effects on sugar metabolism (Nemes-Nagy et al., 2008) have also been reported.

Sugars and fruit acids are important components contributing to the sensory properties and to the consumer acceptance of sea buckthorn berries (Tiitinen, Hakala, & Kallio, 2005; Tiitinen, Yang,

Haraldsson, Jonsdottir, & Kallio, 2006), and the prevalence of these components in the major subspecies of *H. rhamnoides* berries is known (Raffo, Paoletti, & Antonelli, 2004; Tiitinen et al., 2005, 2006; Yang, 2009). The presence of inositols and methylinositols in *H. rhamnoides* has been reported by our laboratory (Kallio et al., 2009; Yang, 2009).

Genetic differentiation between sea buckthorn populations along latitudinal and altitudinal gradients has been observed (Chen, Wang, Zhao, Korpelainen, & Li, 2008; Sheng et al., 2006). Lian, Lu, Xue, and Chen (2000) reported the correlations between latitude, longitude, and altitude and the values of total sugar, total acid, and sugar/acid ratio of *H. rhamnoides* ssp. *sinensis*. However, there is a lack of detailed information on individual sugars and acids and there seems to be that no systematic knowledge on the variations of these compounds in wild sea buckthorn from different growth areas in China is available as yet.

In the present study, wild berries of *H. rhamnoides* ssp. *sinensis* were collected from nine natural growth sites in six provinces in China in order to create an overall profile of the sugars, sugar alcohols, fruit acids, and ascorbic acid in the berries. The growth locations covered the longitude range from 101°23′E to 127°06′E, and the latitude range from 31°01′N to 47°14′N. The altitudes varied from 210 to 3115 m. Special attention was paid to the effects of the latitudes

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and altitudes of the growth locations on the content and composition of these compounds. The berries were collected in three consecutive years to cover the variation among the harvesting years.

2. Materials and methods

2.1. Samples

Wild berries of *H. rhamnoides* ssp. *sinensis* were collected from nine locations in six provinces in China from 2006 to 2008. The growth sites were Suiling Town, Suiling County, Heilongjiang (longitude 127°06′E, latitude 47°14′N, altitude 210 m); West Channel, Liu Jianfang, Dage Town, Fengning County, Hebei (116°34′E, 41°17′N, 818 m); La'erguan, Dongxia Village, Huangyuan County, Xi'ning, Qinghai (101°23′E, 36°45′N, 3115 m); Jiucheng Palace, Hantai Town, Dongsheng District, Ordos, Inner Mongolia (109°48′E, 39°47′N, 1480 m); Beizhe Channel, Li Jiahui, San Daochuan Forestry Centre, Guan Dishan, Lüliang, Shanxi (113°52′E, 37°05′N, 1512 m); Paoma Weir, Xihua Town, Qian'nian Forestry Centre, Guan Dishan, Lüliang, Shanxi (113°52′E, 37°05′N, 2182 m) and three altitudes in Natural Reserve, Wolong, Wenchuan County, Sichuan (106°54′E, 31°01′N, 2000, 2500, and 3000 m). The plants of sea buckthorn in each growth site were divided into two to four field blocks. Berries picked randomly from the plants in the same field block were treated as a single sample lot. Berry samples were harvested in duplicate in Heilongjiang, Inner Mongolia, and Shanxi and in quadruplicate in Hebei and Qinghai. The samples from Sichuan were collected in duplicate in 2007 and in quadruplicate in 2006 and 2008. Berries were picked as soon as they were optimally ripe as defined by experienced horticulturists based on color, flavor, and structure of the berries. Due to the natural variation in these parameters in sea buckthorn, the optimal ripeness of the berries used in the current study was determined by the local sea buckthorn experts, who have been investigating the local sea buckthorn for years and have collected the berry samples for the study. The berries from different growth sites were picked at the same stage of ripeness. The berries were loosely frozen immediately after picking and kept at −20 °C before being analyzed within one year after the collection. The abbreviations *sinensis*-HL, *sinensis*-HB, *sinensis*-QH, *sinensis*-IM, *sinensis*-SX, and *sinensis*-SC will be applied, henceforth, for the berry samples collected from Heilongjiang, Hebei, Qinghai, Inner Mongolia, Shanxi, and Sichuan, respectively.

2.2. Reagents

Reference compounds, D-fructose, quinic acid, and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). D-glucose, *myo*-inositol, and the internal standard D-sorbitol (for sugars) were purchased from Fluka (Buchs, Switzerland). Malic acid and the internal standard tartaric acid (for acids) were purchased from Merck (Darmstedt, Germany). Sucrose and citric acid were purchased from J. T. Baker (Deventer, Holland). L-quebrachitol (1L-2-O-methyl-*chiro*-inositol) was purchased from Alexis Corporation (Läufelfingen, Switzerland).

2.3. Sample preparation

Quadruplicate extractions of sugars, sugar alcohols, fruit acids, and ascorbic acid of each sample were performed as described earlier (Zheng, Yang, Tuomasjukka, Ou, & Kallio, 2009). About 7 g of berries was weighed accurately in duplicate, thawed at room temperature for 15 min, and pressed manually 30 times with a potato masher. The slurry was centrifuged at 4360 ×g for 10 min. The juice was separated and the volume was determined. A portion of 0.25 mL of the juice was taken in duplicate, and 0.25 mL of internal standard sorbitol (0.5 g/100 mL) and 0.25 mL of internal standard tartaric acid (1.0 g/100 mL) were added. The juice was then diluted with distilled water to a final volume of 5 mL. The remnant of the juice was combined, and the pH (Inolab pH level 1 meter, Wissenschaftlich Technische Werkstätten, Weilheim, Germany)

and soluble solids (0 to 32 °Brix refractometer, Atago, Tokyo, Japan) were determined. The diluted juice was filtered (0.45 μm). An aliquot of 300 μL of the filtrate was evaporated to dryness under nitrogen stream at 40 °C and kept in a desiccator over P_2O_5 overnight. Trimethylsilyl (TMS) derivatives of sugars, sugar alcohols, fruit acids, and ascorbic acid were prepared by adding 600 μL of Tri-Sil (Pierce, Rockford, IL, USA) reagent, shaking vigorously with a Vortex (Vortex-Genie, Springfield, MA, USA) for 5 min, and incubating at 60 °C for 30 min. The sample was then cooled down to room temperature.

2.4. Quantification of sugars, sugar alcohols, fruit acids, and ascorbic acid

TMS derivatives of the dried juice samples were analyzed with a Hewlett Packard 5890 Series II GC (Hewlett Packard Co., Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a Hewlett Packard 7673 auto-sampler. The analyses were carried out with a methyl silicone Supelco Simplicity-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm d_f) (Bellefonte, PA, USA). A sample of 1 μL was injected into a split/splitless injector. The flow rate of the carrier gas helium was 1.4 mL/min. The temperature of the injector was 210 °C and that of the detector 290 °C. The column temperature was programmed as 2 min at 150 °C, raised to 210 °C at a rate of 6 °C/min, and to a final temperature of 275 °C at a rate of 40 °C/min, and was held at 275 °C for 10 min. Quantification of the sugars and sugar alcohols was carried out by using sorbitol as an internal standard. The fruit acids and ascorbic acid were quantified by using tartaric acid as an internal standard. Correction factors were determined and used for quantification for each of the sugars, sugar alcohols, fruit acids, and ascorbic acid. The ethyl β-D-glucopyranoside (henceforth ethyl glucose) was quantified as glucose and methyl-*myo*-inositol as L-quebrachitol. The total sugar content was defined as the sum of sugars, sugar derivatives, and sugar alcohols.

2.5. Statistical analysis

Statistical analyses were carried out with SPSS 16.0.1 (SPSS, Inc., Chicago, IL, USA) and Unscrambler 9.8 (Camo Process AS, Oslo, Norway). Differences in the composition between samples from different growth areas and between samples from different altitudes in Sichuan Province, as well as between different harvesting years in each growth sites, were analyzed with a one-way analysis of variance (ANOVA). The Student–Newman–Keuls (SNK) test for population with equal variances and Tamhane's test for population with unequal variances were performed for multiple comparisons. Comparison of samples between two altitudes in Shanxi Province was conducted by an independent-sample *t*-test. Principal component analysis (PCA) and bivariate correlation analysis were combined to investigate the compositional profiles of sea buckthorn berries from different growth areas and the correlation between metabolites in sea buckthorn berries. Bivariate correlation analysis and partial correlation analysis were performed to study the correlation between the latitudes and altitudes of the growth places and the composition of sea buckthorn berries. Differences reaching a confidence level of 95% were considered as statistically significant.

3. Results and discussion

3.1. Compositional profile

Fig. 1 presents the GC–FID chromatograms of the TMS-derivatized berry juice of sea buckthorn collected from Inner Mongolia in 2007 and 2008 and from Sichuan in 2008. Fructose (three isomeric forms of fructose, α- and β-D-furanose and β-D-pyranose) and glucose (two isomeric forms of glucose, α- and β-D-pyranose) were the most abundant sugars in sea buckthorn juice. Malic acid and quinic acid were the two major acids in the berries. The content of ascorbic acid varied from 0.25 to 1.66 g/100 mL juice (Table 2).
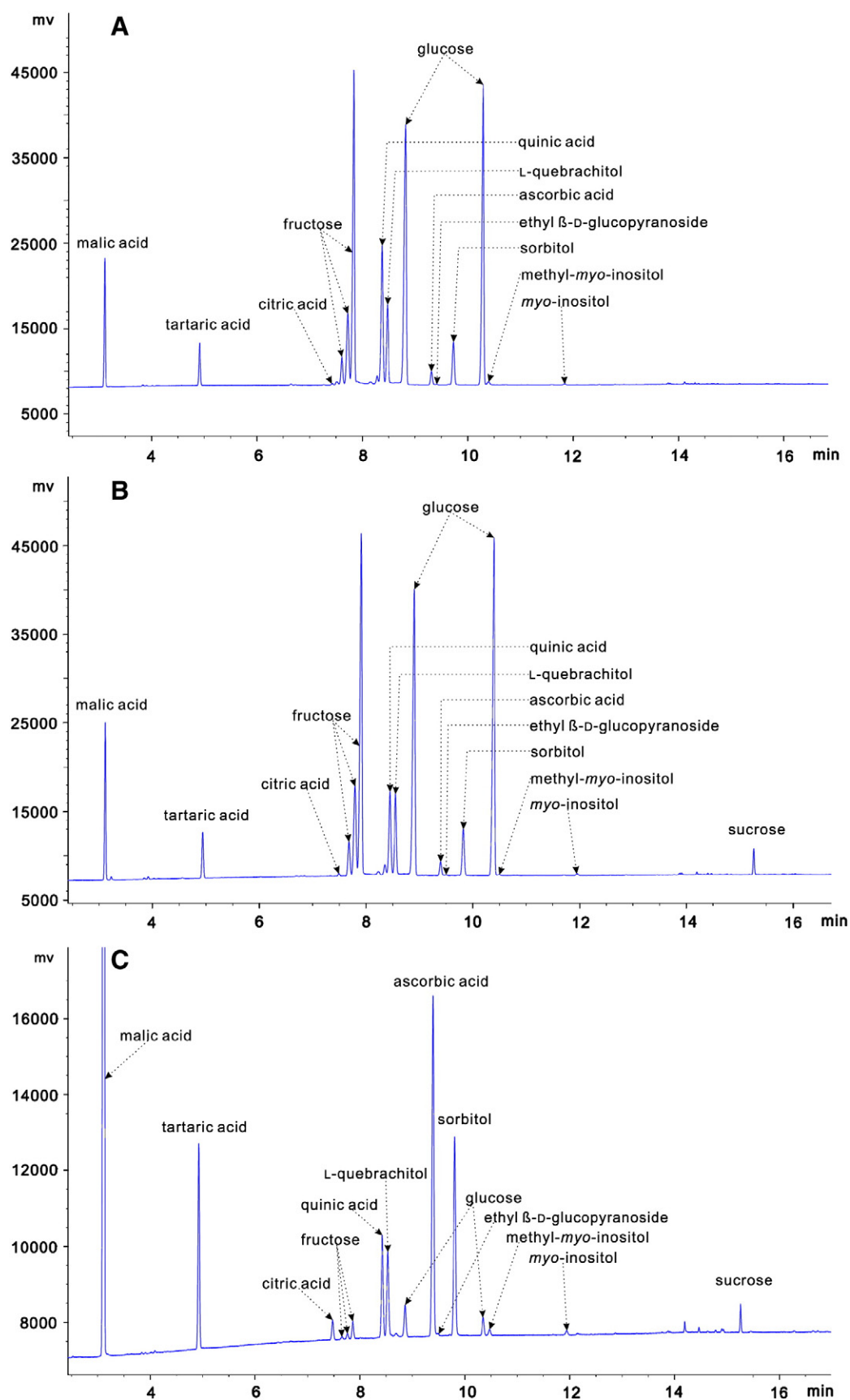
Fig. 1. Gas chromatography–flame ionization detection (GC–FID) chromatograms of sea buckthorn berries (*H. rhamnoides* ssp. *sinensis*) from different regions and harvesting years in China. (A) Sea buckthorn collected from Inner Mongolia in 2007; (B) sea buckthorn collected from Inner Mongolia in 2008; and (C) sea buckthorn collected from Sichuan in 2008.

L-Quebrachitol (1L-2-O-methyl-*chiro*-inositol) was the most abundant sugar alcohol in *H. rhamnoides* ssp. *sinensis*, methyl-*myo*-inositol the second most abundant, and *myo*-inositol the least. No *chiro*-inositol was found in any of the present samples whereas Kallio et al. (2009) reported trace amounts of this compound in some sea buckthorn samples from China.

Trace levels of ethyl glucose were found in all the samples investigated. This is in accordance with the results found in an earlier study by our laboratory (Yang, 2009), which indicated that the berries of *H. rhamnoides* ssp. *sinensis* and *mongolica* contained a trace amount of ethyl glucose. The berries of *H. rhamnoides* ssp. *rhamnoides* contained a much higher amount of this compound than those of ssp. *sinensis* and *mongolica* (Yang, 2009).

The berries from Sichuan with remarkably low sugar contents had totally different chromatogram profiles in comparison with all the other samples (Fig. 1C). The berries from Heilongjiang and Qinghai were the only two samples having no sucrose at all in the samples from all the years studied. The content of sucrose in the berries from other regions varied widely between different harvesting years. For example, sucrose was absent in the berries from Inner Mongolia collected in 2007, whereas the berries collected in 2008 from the same location contained sucrose at a level of 0.31 g/100 mL juice (Fig. 1A and B).

3.2. Comparison of berries from different growth areas

As shown in Tables 1 and 2, the composition of sea buckthorn berries from different growth areas varied widely. The values of fructose, glucose, total sugar, pH, sugar/acid ratio, and sugar/°Brix ratio in berry juice all followed the same order: *sinensis*-IM>*sinensis*-HB>*sinensis*-HL>*sinensis*-SX>*sinensis*-QH>*sinensis*-SC. Among the six growth areas investigated, the berries from Inner Mongolia (*sinensis*-IM) had the highest contents of fructose (1.18–6.41 g/100 mL higher than the berries from other regions, $p<0.05$), glucose (1.67–6.76 g/100 mL higher, $p<0.05$), L-quebrachitol (0.14–0.70 g/100 mL higher, $p<0.05$), and total sugar (2.97–13.94 g/100 mL higher, $p<0.05$) whereas they had the lowest total acid content (0.80–6.47 g/100 mL lower than the berries from other regions, $p<0.05$), hence the highest sugar/acid ratio (1.27–3.66 higher than those from other regions, $p<0.05$). In contrast, samples from Sichuan (*sinensis*-SC) contained the lowest levels of fructose, glucose, L-quebrachitol, quinic acid, total sugar, and sugar/acid ratio and the highest levels of malic acid, citric acid, and ascorbic acid ($p<0.05$). The content of total acid in *sinensis*-SC was significantly higher than all the other samples except *sinensis*-QH. The remarkably low sugar content, especially of fructose (0.08 g/100 mL in *sinensis*-SC vs. 2.83–6.49 g/100 mL in the other samples) and glucose (0.13 g/100 mL vs. 2.82–6.89 g/100 mL), and low sugar/acid ratio (0.07 vs. 0.68–3.73) were clear characteristics of wild sea buckthorn from the Sichuan area. The sugar/acid ratio and the total sugar content are important factors determining the sweetness and the fruity taste of berries and fruits (Poll, 1981; Tang, Kalviainen, & Tuorila, 2001; Tiitinen et al., 2005). Therefore, the wild sea buckthorn berries in Sichuan were expected to be the sourest among all the samples in this study. In contrast, because of the highest sugar content and sugar/acid ratio and the lowest acid content, the berries from Inner Mongolia were supposed to be the sweetest and sensorily the most favored among the berry samples.

In this study, PCA plotting was used to investigate the differences between samples from different growth areas. The closer the samples lay on the plot, the more similar the compositional characteristics of the samples. The PCA biplot of the compositional parameters and the samples is shown in Fig. 2. The first two principal components (PCs) explained 87% of the variance of the data. The PC1 (67%) separated the major components of sugars and sugar alcohols (fructose, glucose, and L-quebrachitol) from malic acid and ascorbic acid. *Sinensis*-HB and *sinensis*-IM had similar characteristics in view of sugar and acid content. They were both highly explained by PC1 as having relatively high contents of fructose, glucose, and L-quebrachitol and low contents

of malic acid and ascorbic acid among the samples investigated. In contrast, *sinensis*-QH and *sinensis*-SC were both characterized as samples containing relatively low contents of sugars but high contents of malic acid and ascorbic acid. The PC2 (20%) represented the minor sugars and sugar alcohols (ethyl glucose, sucrose, methyl-*myo*-inositol, and *myo*-inositol). The PC2 distinguished *sinensis*-IM from *sinensis*-HL. The former had the highest content of sucrose and lowest contents of methyl-*myo*-inositol, *myo*-inositol, and ethyl glucose among all the samples, the latter producing *vice versa* results.

The two PCs also showed a strongly positive correlation between fructose, glucose, and total sugar (correlation coefficients, $r=0.988$, 0.994, and 0.995 between fructose and glucose, fructose and total sugar, and glucose and total sugar, respectively, $p<0.01$). This is in accordance with the finding by Tiitinen et al. (2006) on *H. rhamnoides* ssp. *mongolica* and ssp. *rhamnoides*. However, in contrast to their study, we found significantly negative correlations between fructose and ascorbic acid ($r=-0.775$, $p<0.01$) as well as between glucose and ascorbic acid ($r=-0.762$, $p<0.01$). These discrepancies between the two studies may be due to the genetic differences in the samples. The contents of fructose and glucose in *H. rhamnoides* ssp. *sinensis* also correlated positively with the sugar/acid ratio ($r=0.966$ and 0.959, respectively, $p<0.01$).

3.3. Effect of latitude and altitude on berry composition

Fig. 3 presents certain major compositional parameters of berries collected from growth locations of different altitudes. The latitudes and longitudes of the growth locations are also presented in the figure. Despite some outliers, clearly decreasing trends were seen in the contents of fructose, glucose (Fig. 3A), and methyl-*myo*-inositol (Fig. 3B) as the altitude increased and as the latitude decreased. In contrast, the contents of malic acid (Fig. 3C), ascorbic acid (Fig. 3D), total acid (Fig. 3E), and juice yield (Fig. 3F) increased as the altitude increased and as the latitude decreased. It is important to notice that the concurrent changes in the altitude and the latitude made it difficult to clearly distinguish the effect of the altitude from that of the latitude on the composition of berries.

Bivariate correlation analysis was carried out to investigate the association of altitude and latitude with the composition of the berries. The Spearman's correlation coefficients are presented in Table 3, showing significant correlation between the latitudes/altitudes and most of the compositional parameters analyzed ($p<0.05$). The contents of fructose, glucose, L-quebrachitol, methyl-*myo*-inositol, quinic acid, and total sugar as well as the values of sugar/acid ratio, sugar/°Brix ratio, soluble solids, and pH all correlated positively with the latitude but negatively with the altitude ($p<0.01$). In contrast, the contents of malic acid, citric acid, ascorbic acid, total acid, and juice yield correlated negatively with latitude but positively with altitude ($p<0.01$). It seems that latitude and altitude had opposite effects on the composition of the berries. This is in accordance with the results by Lian et al. (2000), since the same trends were found in contents of total sugar and total acid in response to the growth latitude and the growth altitude. However, in contrast to our study, in their study sugar/acid ratio appeared to correlate positively with the altitude and negatively with the latitude.

Partial correlation analysis (results shown in Table 3) was conducted to investigate the effects of the latitude and altitude on the composition of the berries to exclude interference between latitude and altitude. The opposite effects of latitude and altitude on the composition of the berries found in bivariate correlation analysis were also observed in partial correlation analysis. The contents of fructose, glucose, and total sugar had a strongly positive correlation with the latitude and a weakly negative correlation with the altitude ($p<0.05$). The levels of malic acid, ascorbic acid, and juice yield correlated positively with the altitude and negatively with the latitude ($p<0.05$). The contents of L-quebrachitol and quinic acid were not influenced by the altitude, but had a strongly positive correlation with the latitude ($r=0.792$ and 0.805, respectively,

Table 1
Comparison of sugar and sugar alcohol content in the juice of sea buckthorn berries from different growth sites in China.

Growth site	Altitude	Year	Fructose (g/100 mL)	Glucose (g/100 mL)	Ethyl-glucose (g/100 mL glucose equivalent)	L-quebrachitol (g/100 mL)	Methyl-*myo*-inositol (g/100 mL L-quebrachitol equivalent)	*Myo*-inositol (g/100 mL)	Sucrose (g/100 mL)	Total sugar (g/100 mL)
Heilongjiang	210 m	2006 (n = 8)	2.97 ± 0.12	3.50 ± 0.12	0.03 ± 0.00	0.69 ± 0.03	0.14 ± 0.01	0.07 ± 0.00	N.D.	7.40 ± 0.25
		2007 (n = 8)	5.45 ± 0.96	5.58 ± 1.62	0.02 ± 0.00	0.74 ± 0.22	0.12 ± 0.02	0.03 ± 0.00	N.D.	11.94 ± 2.78
		2008(n = 8)	5.51 ± 0.16	5.30 ± 0.18	0.02 ± 0.00	0.73 ± 0.03	0.16 ± 0.01	0.05 ± 0.00	N.D.	11.76 ± 0.35
		Mean	4.64 ± 1.33[d]	4.79 ± 1.30[d]	0.02 ± 0.01[e]	0.72 ± 0.13[c]	0.14 ± 0.02[c]	0.05 ± 0.02[d]	N.D.[a]	10.37 ± 2.65[d]
Hebei	818 m	2006 (n = 16)	5.84 ± 1.20	5.87 ± 1.01	trace	0.71 ± 0.09	0.10 ± 0.03	0.02 ± 0.00	trace	12.55 ± 2.15
		2007 (n = 16)	5.69 ± 0.61	5.31 ± 0.43	0.01 ± 0.00	1.09 ± 0.07	0.08 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	12.23 ± 0.98
		2008 (n = 12)	4.10 ± 0.54	4.25 ± 0.13	0.02 ± 0.00	0.72 ± 0.14	0.10 ± 0.02	0.02 ± 0.00	0.13 ± 0.03	9.34 ± 0.53
		Mean	5.31 ± 1.13[d]	5.22 ± 0.92[d]	0.01 ± 0.01[bc]	0.85 ± 0.21[d]	0.09 ± 0.02[b]	0.02 ± 0.00[bc]	0.05 ± 0.06[c]	11.56 ± 1.98[d]
Qinghai	3115 m	2006 (n = 16)	2.39 ± 0.36	2.51 ± 0.40	0.04 ± 0.01	0.77 ± 0.09	0.03 ± 0.01	0.01 ± 0.00	N.D.	5.75 ± 0.70
		2007 (n = 12)	2.41 ± 0.18	2.12 ± 0.17	0.01 ± 0.00	0.58 ± 0.04	0.04 ± 0.00	0.01 ± 0.00	N.D.	5.16 ± 0.32
		2008 (n = 8)	4.33 ± 0.19	4.51 ± 0.25	0.01 ± 0.00	0.65 ± 0.05	0.08 ± 0.02	0.02 ± 0.00	N.D.	9.59 ± 0.51
		Mean	2.83 ± 0.86[b]	2.82 ± 0.98[b]	0.02 ± 0.02[de]	0.68 ± 0.11[c]	0.04 ± 0.02[a]	0.01 ± 0.00[a]	N.D.[a]	6.41 ± 1.83[b]
Inner Mongolia	1480 m	2006 (n = 8)	7.17 ± 0.23	7.85 ± 0.05	N.D.	0.90 ± 0.08	0.04 ± 0.01	0.02 ± 0.00	N.D.	15.97 ± 0.18
		2007 (n = 8)	6.88 ± 0.54	7.11 ± 0.30	0.01 ± 0.00	1.09 ± 0.18	0.07 ± 0.01	0.01 ± 0.00	N.D.	15.17 ± 0.90
		2008 (n = 8)	5.41 ± 0.20	5.71 ± 0.20	0.01 ± 0.00	0.99 ± 0.04	0.02 ± 0.00	0.02 ± 0.00	0.31 ± 0.03	12.46 ± 0.42
		Mean	6.49 ± 0.86[e]	6.89 ± 0.93[e]	0.01 ± 0.00[a]	0.99 ± 0.13[e]	0.04 ± 0.02[a]	0.02 ± 0.00[ab]	0.11 ± 0.15[bc]	14.53 ± 1.63[e]
Shanxi	1512 m	2006 (n = 8)	3.76 ± 0.12	5.00 ± 0.53	0.01 ± 0.01	0.64 ± 0.05	0.05 ± 0.01	0.02 ± 0.00	trace	9.48 ± 0.60
		2007 (n = 8)	2.86 ± 0.22	2.82 ± 0.29	0.02 ± 0.00	0.54 ± 0.04	0.11 ± 0.01	0.02 ± 0.00	N.D.	6.38 ± 0.52
		2008 (n = 8)	3.41 ± 0.20	3.90 ± 0.45	0.01 ± 0.00	0.56 ± 0.04	0.09 ± 0.02	0.02 ± 0.00	0.02 ± 0.01	8.01 ± 0.64
	2182 m	2006 (n = 8)	3.32 ± 0.28	4.11 ± 0.28	0.01 ± 0.00	0.56 ± 0.05	0.09 ± 0.02	0.02 ± 0.01	trace	8.12 ± 0.48
		2007 (n = 8)	2.41 ± 0.16	2.84 ± 0.14	0.02 ± 0.00	0.47 ± 0.03	0.07 ± 0.00	0.02 ± 0.00	N.D.	5.84 ± 0.26
		2008 (n = 8)	4.50 ± 0.14	4.47 ± 0.16	trace	0.77 ± 0.12	0.06 ± 0.01	0.02 ± 0.00	0.04 ± 0.01	9.88 ± 0.44
	Average of 1512 m (n = 24)		3.35 ± 0.42[g]	3.91 ± 1.00[g]	0.01 ± 0.01[g]	0.58 ± 0.06[g]	0.08 ± 0.03[g]	0.02 ± 0.00[g]	0.01 ± 0.01[g]	7.95 ± 1.41[g]
	Average of 2182 m (n = 24)		3.41 ± 0.90[g]	3.81 ± 0.74[g]	0.01 ± 0.01[g]	0.60 ± 0.15[g]	0.08 ± 0.02[g]	0.02 ± 0.00[h]	0.02 ± 0.02[g]	7.95 ± 1.73[g]
		Mean	3.38 ± 0.69[c]	3.86 ± 0.87[c]	0.01 ± 0.01[b]	0.59 ± 0.11[b]	0.08 ± 0.03[b]	0.02 ± 0.00[c]	0.01 ± 0.02[b]	7.95 ± 1.56[c]
Sichuan	2000 m	2006 (n = 16)	0.02 ± 0.01	0.09 ± 0.01	0.03 ± 0.00	0.27 ± 0.03	0.05 ± 0.01	0.04 ± 0.00	trace	0.50 ± 0.05
		2007 (n = 8)	0.04 ± 0.00	0.08 ± 0.00	0.02 ± 0.00	0.21 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.46 ± 0.01
		2008 (n = 16)	0.01 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	0.21 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.35 ± 0.03
	2500 m	2006 (n = 16)	0.12 ± 0.01	0.21 ± 0.01	0.02 ± 0.00	0.39 ± 0.03	0.05 ± 0.01	0.03 ± 0.00	trace	0.81 ± 0.05
		2007 (n = 8)	0.21 ± 0.01	0.22 ± 0.01	0.01 ± 0.00	0.33 ± 0.01	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.86 ± 0.02
		2008 (n = 16)	0.09 ± 0.01	0.13 ± 0.01	0.01 ± 0.00	0.32 ± 0.03	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.61 ± 0.03
	3000 m	2006 (n = 16)	0.08 ± 0.01	0.15 ± 0.01	0.02 ± 0.00	0.30 ± 0.02	0.03 ± 0.01	0.02 ± 0.00	trace	0.61 ± 0.05
		2007 (n = 8)	0.09 ± 0.01	0.17 ± 0.01	0.01 ± 0.00	0.39 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.71 ± 0.03
		2008 (n = 16)	0.07 ± 0.01	0.10 ± 0.01	0.01 ± 0.00	0.26 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	0.09 ± 0.01	0.57 ± 0.05
	Average of 2000 m (n = 40)		0.02 ± 0.01[x]	0.07 ± 0.02[x]	0.02 ± 0.01[y]	0.23 ± 0.04[x]	0.04 ± 0.01[y]	0.03 ± 0.02[z]	0.02 ± 0.01[x]	0.43 ± 0.08[x]
	Average of 2500 m (n = 40)		0.12 ± 0.05[z]	0.18 ± 0.04[z]	0.01 ± 0.01[x]	0.35 ± 0.04[z]	0.04 ± 0.02[y]	0.02 ± 0.01[y]	0.02 ± 0.01[x]	0.74 ± 0.12[z]
	Average of 3000 m (n = 40)		0.08 ± 0.01[y]	0.14 ± 0.03[y]	0.01 ± 0.01[x]	0.30 ± 0.05[y]	0.02 ± 0.01[x]	0.01 ± 0.00[x]	0.04 ± 0.04[y]	0.61 ± 0.07[y]
		Mean	0.08 ± 0.05[a]	0.13 ± 0.05[a]	0.02 ± 0.01[cd]	0.29 ± 0.06[a]	0.03 ± 0.01[a]	0.02 ± 0.01[c]	0.03 ± 0.03[c]	0.59 ± 0.15[a]

Means ± standard deviation. Significant differences ($p < 0.05$) are marked as a–e for samples from different growth areas, g–h and x–z for samples from different altitudes in Shanxi and in Sichuan, respectively. Total sugar content was counted as the sum of sugars, sugar derivatives and sugar alcohols. N.D. = not detected.

$p < 0.01$). Latitude and temperature often correlate negatively with each other. Thus the current result supports our findings in another study, in which negative correlations were recognized between the content of L-quebrachitol in *H. rhamnoides* ssp. *sinensis* and the temperature parameters at the growth sites (Yang, Zheng, & Kallio, 2010). In our previous studies on currant cultivars (*Ribes* spp.), the contents of fructose and glucose were found to correlate positively with the growth latitude in the green currant (*R. nigrum* L.) cultivar Vertti and negatively in the black currant cultivars Mortti, Ola and Melalahti (*R. nigrum* L.) and the red currant (*Ribes rubrum* L.) cultivar Red Dutch (Zheng, Kallio, & Yang, 2009; Zheng, Yang, et al., 2009). The relative humidity of the air was found to be among the crucial factors affecting the contents of fructose and glucose in green currant juice, whereas the temperature parameters especially the average temperature in February played a major role in determining the sugar contents in the black and red currant cultivars. Further studies are needed to establish the correlations between the composition of sea buckthorn berries and the weather conditions at the growth places.

In order to gain further insight into the altitudinal effects on the berry composition without interference of the varying longitudes and latitudes, we made separate comparisons between the samples from

the growth locations of three altitudinal levels and the same coordinates in Sichuan and those from the locations of two altitudes and the same coordinates in Shanxi (Tables 1 and 2).

When the altitude increased from 2000 m to 2500 m, the contents of fructose, glucose, L-quebrachitol, and all the acids clearly increased ($P < 0.05$) (Tables 1 and 2, Fig. 3). The content increased from 0.02 to 0.12 g/100 mL for fructose, from 0.07 to 0.18 g/100 mL for glucose, from 0.23 to 0.35 g/100 mL for L-quebrachitol, from 5.30 to 7.34 g/100 mL for malic acid, from 0.04 to 0.09 g/100 mL for citric acid, and from 0.11 to 0.40 g/100 mL for quinic acid. Yet, a further increase in the altitude from 2500 m to 3000 m did not lead to a proportional increase in the content of these compounds in the berries. In fact, some slight decrease was seen in most of these compounds. The samples from the two altitudes in Shanxi did not show a clear difference in most of the parameters measured except for a slightly higher content of quinic acid at an altitude of 1512 m than at 2182 m. However, the small number of samples from Shanxi needs to be considered before any conclusions can be made on the basis of the results.

The effects of the latitudes and altitudes of the growth places on the biosynthetic and the metabolic pathways in plants and therefore

Table 2
Comparison of acids and quality parameters of sea buckthorn berries from different growth sites in China.

Growth site	Altitude	Year	Malic acid (g/100 mL)	Citric acid (g/100 mL)	Quinic acid (g/100 mL)	Ascorbic acid (g/100 mL)	Total acid (g/100 mL)	Sugar/acid	Sugar/°Brix	Soluble solids (°Brix)	pH	Juice yield (mL/100 g)	Berry weight (g/berry)
Heilongjiang	210 m	2006 (n=8)	2.88±0.08	0.07±0.01	2.15±0.07	0.40±0.01	5.49±0.08	1.35±0.03	0.51±0.02	14.5±0.1	2.80±0.03	52.7±0.9	0.20±0.01
		2007 (n=8)	2.19±0.25	0.04±0.01	2.06±0.82	0.25±0.07	4.53±0.51	2.60±0.35	0.66±0.03	18.1±4.7	2.90±0.11	39.7±8.9	0.17±0.05
		2008 (n=8)	2.07±0.07	0.04±0.00	2.66±0.12	0.41±0.03	5.19±0.05	2.27±0.05	0.55±0.02	21.4±0.1	2.85±0.01	38.9±1.6	0.16±0.00
		Mean	2.38±0.39[b]	0.05±0.01[bc]	2.29±0.53[cd]	0.35±0.09[a]	5.07±0.51[b]	2.07±0.58[d]	0.57±0.07[c]	18.0±3.7[bc]	2.85±0.07[c]	43.8±8.1[abcd]	0.18±0.03[d]
Hebei	818 m	2006 (n=16)	2.48±0.62	0.05±0.02	2.94±0.91	0.47±0.27	5.94±0.69	2.13±0.37	0.65±0.07	19.2±1.7	2.79±0.04	41.6±2.1	0.16±0.02
		2007 (n=16)	1.56±0.33	0.03±0.01	2.20±0.29	0.26±0.03	4.04±0.17	3.03±0.29	0.67±0.02	18.2±1.2	2.99±0.03	39.8±4.2	0.09±0.01
		2008 (n=12)	1.76±0.24	0.03±0.01	2.15±0.34	0.48±0.12	4.42±0.27	2.12±0.21	0.54±0.02	17.4±0.6	2.87±0.03	44.2±2.5	0.12±0.02
		Mean	1.95±0.60[a]	0.04±0.02[ab]	2.45±0.69[d]	0.40±0.20[a]	4.84±0.97[b]	2.46±0.53[d]	0.63±0.07[d]	18.3±1.4[c]	2.88±0.09[c]	41.6±3.3[a]	0.12±0.03[ab]
Qinghai	3115 m	2006 (n=16)	8.84±0.45	0.06±0.01	2.60±0.30	1.61±0.13	13.11±0.71	0.44±0.04	0.33±0.01	17.6±1.7	2.35±0.02	49.9±2.9	0.16±0.01
		2007 (n=12)	6.35±0.16	0.05±0.01	1.76±0.20	1.14±0.03	9.30±0.28	0.56±0.03	0.33±0.01	15.5±0.5	2.43±0.01	51.4±0.7	0.16±0.01
		2008 (n=8)	3.86±0.46	0.03±0.00	2.59±0.29	0.64±0.02	7.12±0.35	1.35±0.09	0.46±0.02	20.8±0.3	2.66±0.06	42.5±1.8	0.15±0.01
		Mean	6.91±2.02[d]	0.05±0.01[c]	2.32±0.48[cd]	1.24±0.39[c]	10.51±2.55[e]	0.68±0.37[b]	0.36±0.06[b]	17.6±2.3[bc]	2.44±0.13[a]	48.7±4.1[bc]	0.15±0.01[cd]
Inner Mongolia	1480 m	2006 (n=8)	1.99±0.13	0.03±0.00	2.46±0.41	0.31±0.04	4.79±0.28	3.34±0.16	0.78±0.01	20.6±0.0	2.76±0.03	45.3±0.2	0.11±0.01
		2007 (n=8)	1.98±0.54	0.03±0.01	2.05±0.20	0.36±0.13	4.42±0.84	3.56±0.80	0.75±0.04	20.3±0.4	2.95±0.13	43.8±1.2	0.11±0.00
		2008 (n=8)	1.55±0.06	0.04±0.00	1.05±0.03	0.28±0.03	2.91±0.09	4.28±0.23	0.65±0.01	19.3±0.6	3.17±0.04	42.9±2.1	0.09±0.00
		Mean	1.84±0.37[a]	0.03±0.01[a]	1.85±0.66[bc]	0.32±0.08[a]	4.04±0.96[a]	3.73±0.62[e]	0.72±0.06[e]	20.0±0.7[c]	2.96±0.19[bc]	44.0±1.5[ab]	0.10±0.01[a]
Shanxi	1512 m	2006 (n=8)	2.82±0.04	0.02±0.00	1.64±0.21	0.57±0.21	5.06±0.38	1.89±0.26	0.63±0.06	15.1±0.5	2.71±0.00	50.7±0.8	0.12±0.01
		2007 (n=8)	3.35±0.20	0.03±0.00	2.27±0.15	0.77±0.02	6.43±0.11	0.99±0.08	0.45±0.04	14.1±0.4	2.72±0.06	48.2±0.7	0.13±0.00
		2008 (n=8)	3.52±0.09	0.03±0.00	2.37±0.09	0.73±0.03	6.65±0.20	1.21±0.12	0.46±0.02	17.5±0.7	2.63±0.04	45.8±1.0	0.16±0.00
	2182 m	2006 (n=8)	3.63±0.05	0.02±0.00	1.55±0.23	0.72±0.05	5.93±0.24	1.38±0.14	0.57±0.01	14.3±0.6	2.65±0.01	59.8±2.0	0.14±0.01
		2007 (n=8)	3.95±0.12	0.04±0.00	1.73±0.11	0.71±0.04	6.42±0.21	0.91±0.03	0.43±0.02	13.7±0.2	2.59±0.02	45.3±3.9	0.16±0.02
		2008 (n=8)	2.24±0.05	0.02±0.00	1.81±0.30	0.78±0.12	4.85±0.19	2.04±0.16	0.58±0.01	17.1±0.5	2.84±0.08	50.7±0.1	0.15±0.02
	Average of 1512 m (n=24)		3.23±0.33[g]	0.03±0.01[g]	2.09±0.36[h]	0.69±0.15[g]	6.05±0.76[g]	1.36±0.42[g]	0.51±0.09[g]	15.5±1.6[g]	2.69±0.05[g]	48.2±2.3[g]	0.14±0.02[g]
	Average of 2182 m (n=24)		3.27±0.76[g]	0.03±0.01[g]	1.70±0.24[g]	0.73±0.08[g]	5.73±0.70[g]	1.44±0.49[g]	0.53±0.07[g]	15.0±1.7[g]	2.69±0.13[g]	51.9±6.8[g]	0.15±0.02[g]
		Mean	3.25±0.58[c]	0.03±0.01[a]	1.90±0.37[b]	0.71±0.12[b]	5.89±0.74[c]	1.40±0.45[c]	0.52±0.08[c]	15.3±1.6[b]	2.69±0.09[b]	50.1±5.2[c]	0.14±0.02[bc]
Sichuan	2000 m	2006 (n=16)	5.94±0.45	0.04±0.01	0.14±0.02	1.40±0.15	7.52±0.61	0.07±0.00	0.07±0.01	7.5±0.2	2.36±0.04	51.6±3.1	0.09±0.01
		2007 (n=8)	4.94±0.11	0.04±0.00	0.13±0.00	0.86±0.02	5.97±0.13	0.08±0.00	0.06±0.00	7.6±0.0	2.48±0.00	52.7±0.5	0.12±0.00
		2008 (n=16)	4.84±0.26	0.04±0.00	0.07±0.00	1.09±0.04	6.04±0.30	0.06±0.00	0.05±0.00	7.0±0.3	2.34±0.04	62.7±1.0	0.12±0.00
	2500 m	2006 (n=16)	8.37±0.48	0.13±0.05	0.63±0.08	1.66±0.11	10.80±0.56	0.07±0.00	0.07±0.00	10.9±0.3	2.45±0.01	53.3±3.7	0.11±0.00
		2007 (n=8)	6.33±0.21	0.06±0.00	0.29±0.01	0.82±0.03	7.49±0.26	0.11±0.00	0.08±0.00	10.3±0.1	2.42±0.01	54.6±2.5	0.18±0.00
		2008 (n=16)	6.81±0.22	0.07±0.00	0.22±0.02	0.99±0.04	8.10±0.27	0.07±0.00	0.06±0.00	9.7±0.4	2.34±0.04	57.6±1.2	0.16±0.01
	3000 m	2006 (n=16)	6.66±0.70	0.06±0.01	0.48±0.05	1.39±0.06	8.59±0.81	0.07±0.00	0.06±0.00	9.8±0.3	2.45±0.01	53.6±2.1	0.12±0.01
		2007 (n=8)	6.26±0.15	0.06±0.00	0.34±0.01	0.82±0.02	7.48±0.18	0.09±0.00	0.08±0.00	9.4±0.0	2.43±0.01	59.1±0.0	0.17±0.01
		2008 (n=16)	6.73±0.41	0.07±0.00	0.26±0.02	1.28±0.07	8.35±0.49	0.07±0.00	0.06±0.00	9.9±0.3	2.36±0.01	58.9±1.6	0.16±0.01
	Average of 2000 m (n=40)		5.30±0.62[x]	0.04±0.00[x]	0.11±0.04[x]	1.17±0.23[x]	6.62±0.86[x]	0.07±0.01[x]	0.06±0.01[x]	7.3±0.4[x]	2.38±0.06[x]	56.2±5.9[x]	0.11±0.02[x]
	Average of 2500 m (n=40)		7.34±0.94[z]	0.09±0.04[z]	0.40±0.20[y]	1.23±0.37[x]	9.06±1.51[z]	0.08±0.02[y]	0.07±0.01[z]	10.3±0.6[z]	2.40±0.06[x]	55.3±3.2[x]	0.15±0.03[y]
	Average of 3000 m (n=40)		6.61±0.54[y]	0.07±0.01[y]	0.36±0.11[y]	1.23±0.22[x]	8.27±0.72[y]	0.07±0.01[y]	0.06±0.01[y]	9.8±0.3[y]	2.41±0.04[x]	56.8±3.2[x]	0.15±0.02[y]
		Mean	6.42±1.11[d]	0.07±0.03[d]	0.29±0.18[a]	1.21±0.28[c]	7.98±1.48[d]	0.07±0.01[a]	0.06±0.01[a]	9.1±1.4[a]	2.39±0.06[a]	56.1±4.2[d]	0.13±0.03[b]

Means ± standard deviation. Significant differences ($p < 0.05$) are marked as a–e for samples from different growth areas, g–h and x–z for samples from different altitudes in Shanxi and in Sichuan, respectively.
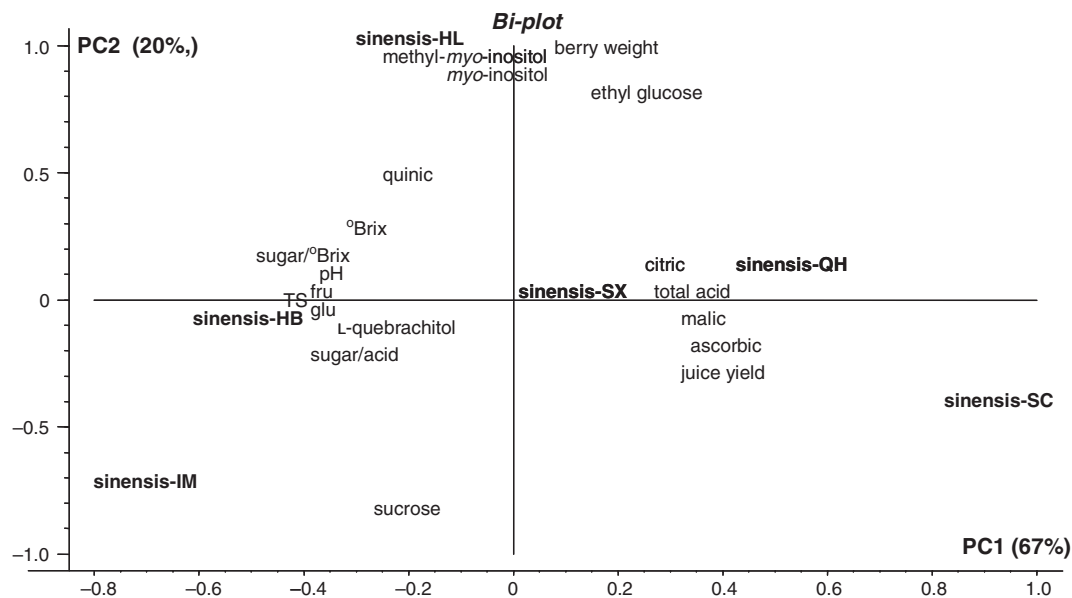
Fig. 2. Differences in PCA between sea buckthorn berries collected from different growth areas. *Sinensis*-HL, *sinensis*-HB, *sinensis*-QH, *sinensis*-IM, *sinensis*-SX, and *sinensis*-SC were abbreviations of sea buckthorn samples collected from Heilongjiang, Hebei, Qinghai, Inner Mongolia, Shanxi, and Sichuan, respectively. Fru, fructose; glu, glucose; TS, total sugar; malic, malic acid; citric, citric acid; quinic, quinic acid; ascorbic, ascorbic acid.

the fruit composition are the results of the complex effects of growth environments such as soil conditions and climatic conditions as well as the interaction between the genetic and the environmental factors. Light intensity, photoperiod, and temperature respond markedly to variation in latitude and altitude. These factors strongly influence the metabolism and concentration of metabolites in plants and fruits (Buchanan et al., 2000). In addition to temperature and radiation, water supply and air humidity are also important factors affecting the composition of sugars and acids in berries and fruits (De Pascale, Martino, Raimondi, & Maggio, 2007; Miller, Smith, Boldingh, & Johansson, 1998; Zheng, Kallio, et al., 2009; Zheng, Yang, et al., 2009).

In the study on *H. rhamnoides* ssp. *sinensis* by random amplified polymorphic DNA (RAPD) markers by Sheng et al. (2006), the results of the multiple regression analysis indicated that genetic distance had a significant correlation ($p<0.05$) with altitudinal and latitudinal distances among the populations. However, the Mantel test in their study showed that genetic distance had only a significant correlation with altitudinal distance ($p<0.05$). Chen et al. (2008) investigated the genetic diversity of sea buckthorn samples at varying altitudes from the same area (Natural Reserve, Wolong, Sichuan) as were the berries used in our study by using inter-simple sequence repeats (ISSR) markers. They revealed substantial genetic divergence present among populations and significant correlations between altitudinal distance and genetic distance. This indicates that altitudinal gradients may be the prime cause affecting the genetic variation pattern of different populations in *H. rhamnoides* ssp. *sinensis*. Such variation according to the growth altitudes may be caused by complex topography, featuring tall, zigzag positioned mountains, which effectively restrict gene flow. The altitudinal variation found in the genetic background may also have been the result of long adaptation to the different climatic conditions at different altitudes, which produce varying selective pressure, e.g. delayed flower development.

3.4. Differences between berries from different harvesting years

Significant variations ($p<0.05$) between different harvesting years were found in the contents of fructose, glucose, ethyl glucose, malic acid, citric acid, total sugar, and total acid as well as the values of sugar/acid ratio and sugar/°Brix ratio in all the samples from different

regions. This is in agreement with the earlier findings by our research group (Tiitinen et al., 2005, 2006; Yang, 2009). The pattern of variation with harvesting years differed among the different regions. Taking sugars as an example, the contents of fructose and glucose were significantly higher ($p<0.05$) in the berries of *sinensis*-HL and *sinensis*-QH collected in 2008 than those collected in 2006, while the situation was *vice versa* in berries of *sinensis*-HB and *sinensis*-IM.

The berries from Qinghai showed the biggest year-to-year variation among all the samples studied. The contents of fructose, glucose, and total sugar in berries from Qinghai collected in 2008 reached 1.8, 2.1, and 1.9 times of the corresponding levels in the berries collected in 2007, respectively ($p<0.05$). The contents of malic acid, citric acid, ascorbic acid, and total acid in berries collected in 2006 were 2.3, 2.0, 2.5, and 1.8 times as high as the levels in those collected in 2008 in Qinghai, respectively ($p<0.05$). The sugar/acid ratio in the samples collected from Qinghai in 2008 was 3.1 and 2.4 times as high as the values in those collected in 2006 and 2007 respectively, from the same location ($p<0.05$). However, significant differences ($p<0.05$) in juice yield between harvesting years were only observed in berries collected from Sichuan at the altitudes of 2000 m and 3000 m among all the samples studied. No significant difference ($p>0.05$) between harvesting years was found in the content of L-quebrachitol in berries from Heilongjiang, nor in the content of methyl-*myo*-inositol in berries from Hebei and Qinghai. The content of ascorbic acid in berries from Inner Mongolia and from Shanxi at an altitude of 2182 m was not influenced ($p>0.05$) by harvesting years either.

Because the samples were picked randomly from different bushes within populations, the annual variation of the compositional parameters of berries was probably due to both the variation of weather conditions in different years and intra-population variation between bushes. The considerable annual variation of compositional parameters suggested that the evaluation of raw materials should be conducted every year prior to industrial processing of sea buckthorn.

4. Conclusion

The composition and content of sugars, sugar alcohols, fruit acids, and ascorbic acid of the berries of *H. rhamnoides* ssp. *sinensis* vary greatly
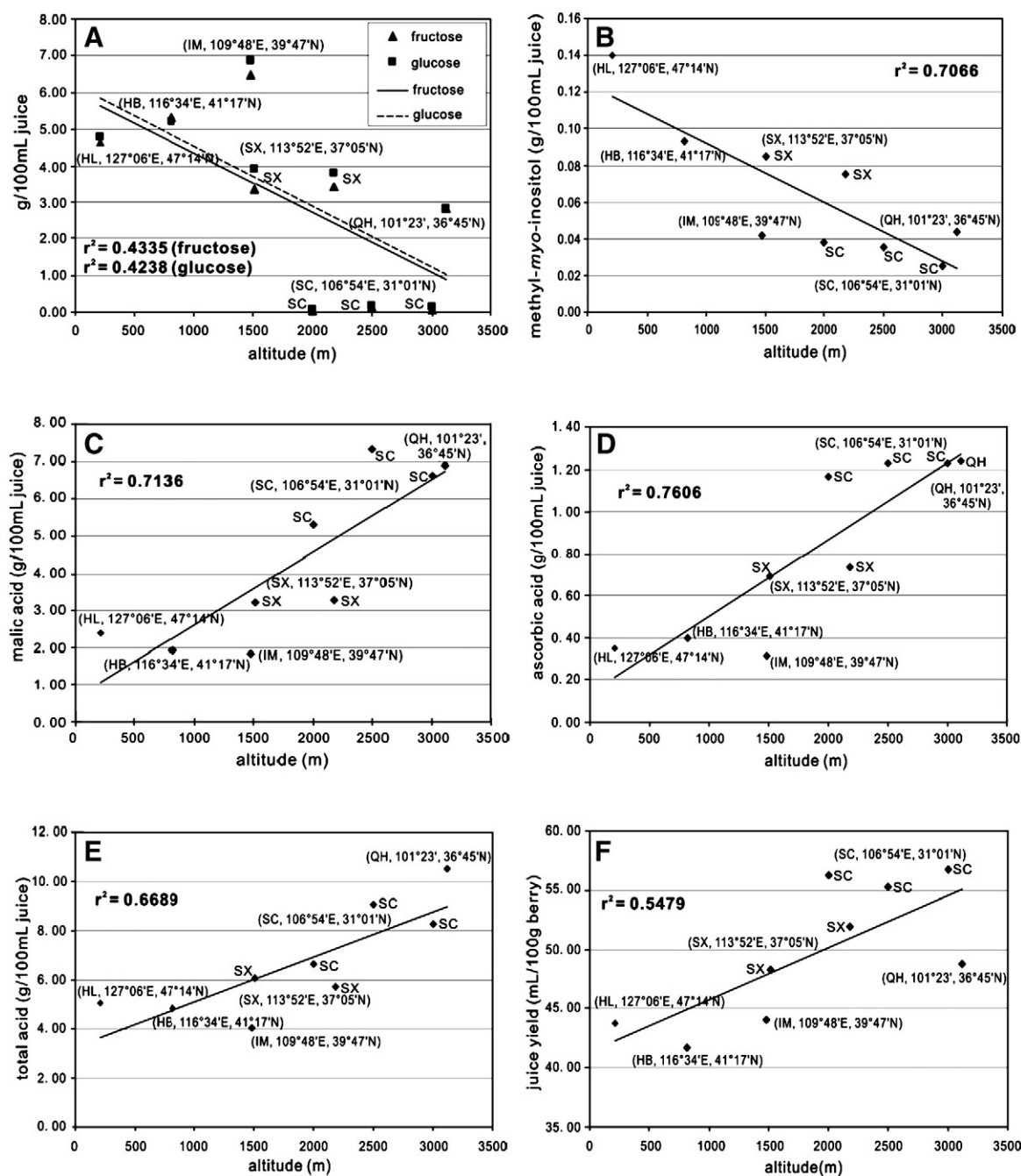
Fig. 3. Correlations between spatial parameters and contents of fructose and glucose (A), methyl-*myo*-inositol (B), malic acid (C), ascorbic acid (D), total acid (E), and juice yield (F). Growth area, longitude, and latitude were indicated in parentheses on the plot. HL, Heilongjiang; HB, Hebei; IM, Inner Mongolia; SX, Shanxi; SC, Sichuan; and QH, Qinghai.

with growth locations. The changes in the berry composition were oppositely associated with the variation in latitudes and altitudes. The contents of fructose, glucose, and total sugar correlated positively with the growth latitude but negatively with the altitude. In contrast, the contents of malic acid and ascorbic acid correlated negatively with the latitude but positively with the altitude. The contents of L-quebrachitol and quinic acid had a strongly positive correlation with the latitude. The influence of spatial parameters on composition of berries may be explained by combinations of complex environmental factors, which deserve to be investigated further. This study provides useful guidelines for berry breeding and cultivation as well as industrial utilization of sea buckthorn.

Table 3
Correlation coefficients between the compositional parameters of sea buckthorn berries and the latitude and altitude of growth sites.

	Malic acid	Citric acid	Quinic acid	Ascorbic acid	Fructose	Glucose	Ethyl glucose	L-quebrachitol	Methyl-myo inositol	Myo-inositol	Sucrose	Total sugar	Total acid	Sugar/acid	Sugar/°Brix	°Brix	pH	Juice yield	Berry weight
Spearman's correlation coefficients																			
Latitude	−0.812**	−0.550**	0.777**	−0.813**	0.882**	0.869**	−0.093	0.826**	0.724**	0.260**	−0.147*	0.879**	−0.710**	0.899**	0.883**	0.811**	0.837**	−0.770**	−0.002
Altitude	0.820**	0.454**	−0.319**	0.749**	−0.555**	−0.547**	0.062	−0.434**	−0.632**	−0.431**	−0.130*	−0.556**	0.821**	−0.610**	−0.605**	−0.388**	−0.725**	0.544**	0.234**
Partial correlation coefficients																			
Latitude	−0.278*	−0.241*	0.805**	−0.370**	0.793**	0.767**	0.102	0.792**	0.359*	−0.155	0.018	0.794**	−0.163	0.622**	0.789**	0.871**	0.545**	−0.610**	0.266*
Altitude	0.472**	0.153	0.114	0.339**	−0.313**	−0.268**	0.040	−0.171	0.039	−0.059	−0.225	−0.297**	0.479**	−0.408**	−0.206	0.068	−0.325**	0.282*	0.569**

* $p < 0.05$.
** $p < 0.01$.

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